

United States Patent Application for

COMPOUNDS AND METHOD FOR ENHANCING THE EFFICACY OF ANTI-CANCER DRUGS


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COMPOUNDS AND METHOD FOR ENHANCING THE EFFICACY OF ANTI-CANCER DRUGS

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FIELD OF INVENTION

This invention relates to polyamine compounds and more particularly to their use
as anticancer agents and their use as a vector for the enhancement of anti-cancer drug
15 activity.

BACKGROUND AND PRIOR ART

One of the major shortcomings of current cancer therapies is the non-selective
delivery of the antineoplastic drug to both targeted tumor cells and healthy cells.
Enhanced selectivity of such drugs could diminish their associated toxicity by reducing
20 their uptake by healthy cells. Moreover, selective delivery would increase drug potency
by lowering the effective dosage required to kill the affected cell type. Vectored systems,
which have enhanced affinity for cancer cells would be an important advance in cancer
therapy.

Polyamines are naturally occurring amines, which form polycations in vivo. These stabilize DNA architectures and are cellular growth factors. All cells contain some form of the native polyamines: putrescine, spermidine or spermine. Rapidly dividing cells (such as cancer cells) require large amounts of polyamines, and cells can either
5 biosynthesize or import these essential growth factors. Many tumor cell lines have been shown to have very high levels of polyamines and an active polyamine transporter.

Polyamine structures have been exploited for use in various drug strategies, such as demonstrated in United States Patents: Bergeron, 6,342,534 and 5,866,613; Prakash, 5,109, 024; Iwata 6,319,956 and Published application 2002/0067472 A1; Bowlin
10 5,719,193; and, Klosel 6,281,371 B1 and published document "A Comparison of Structure-Activity Relationships between Spermidine and Spermine Analogue Antineoplastics," by Bergeron, R. J.; Feng, Y.; Weimar, W. R.; McManis, J. S.; Dimova, H.; Porter, C.; Raisler, B.; Phanstiel IV, O. *J. Med. Chem.* **1997**, 40, No. 10, 1475-1494.

Bergeron 6,342,534 should be considered with respect to Column 3, lines 51-67
15 and Column 4, lines 39-48, Table 1 and Table 2, in which the emphasis is on bis-substituted tetraamine systems terminated with N-ethyl, N-piperidinyl, and pyridinyl units. Bowlin referred to above should be considered with respect to Column 1, lines 51-67 in which is described, compounds useful for potentiating the immune response. Bowlin's compounds are limited to activating cells to be killed by the immune system.
20 Thus, Bowlin requires an immune system to work with their disclosed drugs.

Other research in using polyamine conjugates for cellular entry has been described in published documents (Cohen, G. M.; Cullis, P.; Hartley, J. A.; Mather, A. Symons, M. C. R.; Wheelhouse, R. T. Targeting of Cytotoxic Agents by Polyamines:

Synthesis of a Chloroambucil-Spermidine Conjugate. *J. Chem. Soc. Chem. Commun.* **1992**, 298-300; Cullis, P.M.; Merson-Davies, L.; Weaver, R. Conjugation of a polyamine to the bifunctional alkylating agent chlorambucil does not alter the preferred cross-linking site in duplex DNA. *J. Am. Chem. Soc.* **1995**, *117*, 8033-8034; Phanstiel IV, O.;
5 Price, H.L.; Wang, L.; Juusola, J.; Kline, M.; Shah, S.M. The Effect of Polyamine Homologation on the Transport and Cytotoxicity Properties of Polyamine-(DNA-Intercalator) Conjugates. *J. Org. Chem.* **2000**, *65*, 5590-5599; Wang, L.; Price, H.L.; Juusola, J.; Kline, M.; Phanstiel, IV, O. "Influence of Polyamine Architecture on the Transport and Topoisomerase II Inhibitory Properties of Polyamine DNA-Intercalator
10 Conjugates," *J. Med. Chem.* **2001**, *44*, 3682-3691; Delcros, J-G.; Tomasi, S.; Carrington, S.; Martin, B.; Renault, J.; Blagbrough, I.S.; Uriac, P. Effect of spermine conjugation on the cytotoxicity and cellular transport of acridine. *J. Med. Chem.*, **2002**, *45*, 5098-5111).

The prior art by Cullis et al is limited to delivering a DNA-alkylating agent (chlorambucil) to cells using spermidine, a non-optimal polyamine vector. The
15 chlorambucil substituent is linked via a tether to the internal N⁴-nitrogen of the spermidine chain. Recent findings have shown this internal N-alkylation motif used by Cullis to be a less than optimal arrangement for using the polyamine transporter. The previous publications by Phanstiel IV et al are limited to branched polyamine systems built from spermine and spermidine platforms, again using non-optimized polyamine
20 vectors. The report by Blagbrough et al focused on using tetraamine derivatives of spermine to deliver acridine to cells. Blagbrough's compounds are limited by the use of less than optimal spermine vectors to deliver a less potent acridine drug into cells.

Since the 1980s several laboratories have probed the transport properties of polyamines into various cell types (*E. coli*, yeast and mammals). The polyamine transporter in *E. coli* is perhaps the best understood as the transporter gene and several protein gene products (Pot A-F) have been identified. In particular the PotB and PotC
5 proteins form a trans-membrane channel, which facilitates polyamine transport. PotD is a periplasmic, polyamine-binding protein, which prefers spermidine over putrescine. Moreover, the X-ray crystal structure of spermidine bound to PotD revealed that the molecular recognition events involved in spermidine binding is controlled by specific amino acid residues and a bound water molecule. Specifically, through this water
10 molecule, the bound spermidine molecule forms two hydrogen bonds with Thr 35 and Ser 211. In a related study the PotF protein was shown to selectively bind putrescine. The PotF crystal structure, in combination with the mutational analysis, revealed the residues crucial for putrescine binding (Trp-37, Ser-85, Glu-185, Trp-244, Asp-247, and Asp-278) and the importance of water molecules for putrescine recognition. Therefore, the *E.coli*
15 studies provided a striking example of how cells can discriminate between structurally similar di-and tri-amine substrates, (e.g., putrescine (PUT) and spermidine (SPD), respectively). While significant work has also been accomplished in yeast and other systems, the proteins involved in mammalian polyamine transport have not yet been isolated and characterized beyond a kinetic description. Clearly, the lack of structural
20 detail associated with the mammalian polyamine transporter is a glaring void in the knowledge base.

SUMMARY OF THE INVENTION

The first objective of the present invention is to provide compounds that are useful as anti-cancer agents.

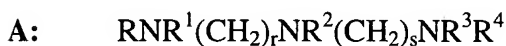
The second objective of the present invention is to provide a method for
5 enhancing the efficacy of anti-cancer agents.

The third objective of the present invention is to provide a method for preparing the anti-cancer compounds.

The fourth objective of the present invention is to provide compounds and methods for treating cancer cells without requiring the immune system.

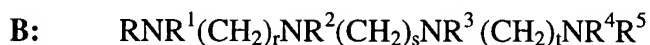
10 Preferred embodiments of this invention include:

Compounds of the formula **A**,



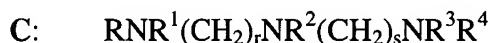
15 or a pharmaceutically acceptable salt thereof, wherein R is selected from the group consisting of naphthylmethyl, naphthylethyl, anthracenylmethyl, anthracenylethyl, pyrenylmethyl, R¹, R², R³, and R⁴ are selected from at least one of hydrogen, alkyl, cycloalkyl, alkylaryl, para-toluenesulfonyl, arenesulfonyl, alkylsulfonyl, acyl, carbamoyl, and r is 2-18 and s is 2-18;

20 Compounds of the formula **B**,



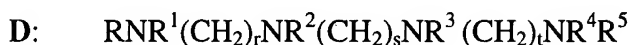
or a pharmaceutically acceptable salt thereof, wherein R is selected from the group consisting of naphthylmethyl, naphthylethyl, anthracenylmethyl, anthracenylethyl, pyrenylmethyl, wherein R¹, R², R³, R⁴ and R⁵ are selected from at least one of the following: hydrogen, alkyl, cycloalkyl, alkylaryl, para-toluenesulfonyl, arenesulfonyl, alkylsulfonyl, acyl, carbamoyl and r is 2-18, s is 2-18 and t is 2-18;

Compounds of the formula C,



or a pharmaceutically acceptable salt thereof, where R is a chemotherapeutic agent and R¹-R⁴ are at least one of hydrogen, alkyl, acyl, carbamoyl or alkylaryl, and r is 2-18, and s is 2-18;

A compound of the formula D,



or a pharmaceutically acceptable salt thereof, where R is a chemotherapeutic agent and R¹-R⁵ are at least one of hydrogen, alkyl, acyl, carbamoyl or alkylaryl, and r is 2-18, s is 2-18 and t is 2-18; their use in pharmaceutical compositions; and their methods of fabrication.

Further objects and advantages of this invention will be apparent from the following detailed descriptions of the presently preferred embodiments, which are illustrated schematically in the accompanying drawings.

BRIEF DESCRIPTION OF THE FIGURES

Figure 1 is a sample conjugate system with two examples, triamine 8 and tetraamine 13.

Figures 2A, 2B and 2C (Schemes 1-4) provide general method schemes for the synthesis of the compounds of the invention.

Figure 3 is a general strategy to enhance cell uptake by polyamine vectoring systems.

Figure 4 provides examples of an existing anti-cancer drug doxorubicin substituted with either a triamine, e.g., **14** (or a tetraamine, e.g., **15**) to improve its chemotherapeutic potency.

- 5 Figure 5 (Scheme 5) - illustrates a synthetic method to attach the polyamine vector onto an existing chemotherapeutic, doxorubicin. This methodology can lead to the synthesis of the polyamine-doxorubicin conjugate **14**.

Figures 6A-6B (Schemes 6 and 7) - illustrates the synthesis of various aryl substituted polyamines (**27a**, **27b**, **8e**, **27d**), a hydroxylated polyamine derivative **30** and a
10 cyclohexyldiamine analogue **31**.

Figure 7 illustrates the structures of **32-35** as examples of systems with altered amine scaffolds appended to an anthracenyl methyl unit.

Figure 8 illustrates a naphthylethyl triamine derivative, **36**.

Figure 9 illustrates an anthracenylethyl triamine derivative, **37**.

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DESCRIPTION OF THE PREFERRED EMBODIMENTS

Before explaining the disclosed embodiments of the present invention in detail it is to be understood that the invention is not limited in its application to the details of the particular arrangements shown, since the invention is capable of other embodiments.

- 20 Also, the terminology used herein is for the purpose of description and not of limitation.

It would be useful to discuss the definitions of some words used herein and their applications before discussing the invention, which provides compounds and methods for treating cancer cells without requiring the immune system for their cytotoxicity. For the

purposes of this patent application, the term “vector” is used to denote a special chemical message, which is recognized by the polyamine transport system of cells. This special chemical message is a polyamine with the proper or ideal spacing units in between the nitrogen centers. Research has shown that having the proper spacing unit (distance
5 between the nitrogen centers) and having the appropriate number of positive charges (via protonation of the nitrogen centers) is critical for selective cellular uptake.

- 1) “Vectored systems” relates to polyamine conjugates, which have a polyamine message which is recognized by the polyamine transport system on the surface of cells and have enhanced uptake into
10 cells with highly active polyamine transporters over those which do not, (e.g., CHO vs. CHO-MG cells).
- 2) The term “transporter” is used to describe the cellular process of binding and/or importing a chemical entity, which is outside the cell. The chemical entity in this case is the polyamine conjugate, i.e. a polyamine scaffold
15 covalently attached to a toxic agent.
- 3) The term “conjugate” is used to describe a polyamine architecture, which is covalently bound to a cytotoxic agent (e.g., an anthracenyl methyl unit) or to a known chemical agent with anti-cancer properties, e.g., doxorubicin.
- 20 4) “Cell selectivity” denotes the ability of a polyamine conjugate to selectively enter cells with highly active polyamine transporters (e.g. CHO cells or B16 melanoma cells) over those that have lower polyamine transport activity (e.g. CHO-MG cells or Mel-A cells).

- 5) "IC₅₀ value" is the concentration of drug needed to kill 50% of the treated cell population. The lower the value the more cytotoxic the drug is to that cell type.
- 6) "K_i value" reflects the affinity of the drug architecture for the polyamine transporter. The lower the value of the K_i the higher the affinity of the drug for the polyamine transporter.
- 7) L1210 cells are mouse leukemia cells and are a standard well-used benchmark for evaluating cytotoxicity of new drug systems, especially polyamine containing drugs.
- 8) Chinese hamster ovary cells (CHO cells) have an active polyamine transporter. This cell type is very susceptible to drugs, which use the polyamine transporter to gain access to cells (i.e. polyamine conjugates),.
- 9) Chinese hamster ovary cells, which are chemically mutated to be polyamine transport-deficient will be referred to as the CHO-MG cell line. This cell type should have lower susceptibility to polyamine conjugates, which use the polyamine transporter to gain access to cells, since it does not have an active transporter to facilitate their uptake.
- 10) B16 cells are melanoma, skin cancer cells with highly active polyamine transporters. These cells should be very susceptible to polyamine conjugates which use the polyamine transporter to gain access to cells.
- 11) Mel-A cells are normal melanocytes, skin cells, which have moderately active polyamine transporters. These cells should be moderately susceptible to polyamine-conjugates, but less so than the B-16 cells.

This invention has identified polyamine compounds, which have surprising cytotoxicity, unexpected selectivity in killing cancer cells (or cells with active polyamine transporters), and/or facilitate the delivery of known toxic agents into cancer cells.

A panel of amine substrates of the general formula indicated in Figure 1 (i.e., those of general type **8** and **13** as well as other systems to be discussed later) were tested for efficacy in cells containing active and deficient polyamine transporters (i.e., Chinese hamster ovary CHO cells and CHO-MG cells, respectively). In addition, murine leukemia (L1210) cells and L1210 cells pretreated with DFMO (Difluoromethylornithine, an inhibitor of ornithine decarboxylase) were also treated with the polyamines illustrated in Figure 1 (i.e. those of general type **8** and **13**) as well as other systems to be discussed later. The results indicate micromolar concentration IC_{50} values in three cell lines along with varying K_i values (a transporter affinity measure) and are provided in Tables 1 and 2. Table 1 illustrates the fact that different polyamine compounds have different cytotoxicity profiles, e.g., the IC_{50} value varies from 0.3 to 36.3 μ M in the L1210 cell study (Table 1, column 2). The presence of DFMO is known to enhance polyamine uptake into L1210 cells and should make the cells more sensitive to the tested polyamine analogues. Indeed, the IC_{50} values were typically lower in the presence of DFMO (Table 1, column 3). This finding is consistent with certain analogues using the polyamine transporter to gain access to these cells. The distance separating the nitrogen centers was directly related to cytotoxicity. Attaching the anthracenylmethyl subunit to a particular polyamine “vector” motif provided enhanced cytotoxicity. The structures of compounds **8b-8g** are defined in Figure 2, Scheme 2. Note: the term “3,3-triamine” refers to the fact that molecule **8b** has two three carbon spacer units separating the three nitrogen centers.

For example in Table 1, **8b**, a conjugate bearing the “3,3-triamine” motif was only 1.3 fold more toxic in the presence of DFMO, whereas the related 4,4-triamine motif **8e** was twice as toxic. This effect was even more dramatic with tetraamine systems **13e** and **13f**, which were 7.2 and 4.7 fold more toxic, respectively, in the presence of DFMO.

5 Note the control compounds **34** and **35**, which do not contain the proper polyamine vector, gave lower cytotoxicity in the presence of DFMO (both were 0.7 fold less toxic). These results suggested that the observed cytotoxicity enhancement with DFMO is related to the ability of the conjugate to utilize the polyamine transport system.

As shown in Table 2, these same controls **34** and **35** were not very selective in

10 killing CHO and CHO-MG cells as they had similar toxicities in both lines. Indeed, specific structures of the polyamine component were needed for the desired cell selectivity. The triamines of the invention have demonstrated over 150-fold higher cytotoxicity in killing cells with active polyamine-transporters (as modeled by the CHO line) over cells, which are polyamine-transporter deficient (as modeled by the CHO-MG

15 cell line). For example, the 4,4-triamine conjugate **8e** was one of the most selective compounds and was very toxic to CHO cells ($IC_{50} = 0.45 \mu M$), but much less so to the CHO-MG cell line ($IC_{50} = 66.7 \mu M$).

While a few investigators have successfully “ferried” polyamine-drug conjugates into cells, limited systematic studies have been conducted on the mono-substituted linear

20 polyamines as vector systems. This, in part, may stem from their less direct syntheses, which involve several steps. The method of synthesis of some of the triamines (and tetraamines) of the invention are provided in examples below and in Figures 2A, 2B, 2C, 5 and 6 (Schemes 1-7).

The initial conjugates are comprised of an anthracene nucleus covalently bound to a polyamine framework. The anthracene component was selected due to significant preliminary data, which revealed its increased potency over an acridine analogue in murine leukemia (L1210) cells. Moreover, the anthracene provides a convenient UV
5 “probe” for compound isolation (and identification) and elicits a toxic response from cells upon entry (presumably through DNA coordination). The uptake of several anthracene-polyamine conjugates by the polyamine transporter (PAT) has been demonstrated. The large size and sweep volume of the appended anthracene system suggests that other architectures (e.g., doxorubicin) may also be conveyed into cells via this transporter. In
10 fact, compound **27d**, a pyrene derivative, is also selectively imported into cells with active polyamine transporters. Therefore, while the anthracene component offers a convenient probe for polyamine delivery studies, the invention is not limited thereto.

To optimize delivery, polyamine architectures are needed, which facilitate uptake via the polyamine transporter (PAT). The polyamine tail has been shown to facilitate
15 uptake, impart water solubility to the conjugate, and enable dosing as aqueous solutions.

Virtually all cells contain substantial amounts of at least one of the polyamines: putrescine (PUT), spermidine (SPD), or spermine (SPM). Since the polyamine component represents an important cellular “feedstock”, one would expect preferential uptake by rapidly dividing cells. Polyamines are a requirement for the optimum growth
20 and replication of various cell types and are present in higher concentrations in rapidly proliferating cells. The fact that polyamines can be taken up by tissues from the circulation is known, since the metabolism of labeled polyamines has been studied

rapidly dividing cells) take up exogenous polyamines in increased amounts via a specific transport system. The high specific activity of polyamine transport in tumor cells is thought to be associated with the inability of biosynthetic enzymes to provide sufficient levels of polyamines to sustain the rapid cell division. These “bio-production”
5 constraints are partially offset by scavenging polyamines from exogenous sources.

Additional studies have indicated that polyammonium cations (PACs) have a very high DNA affinity, but are loosely bound and can “read” DNA very rapidly because of their otherwise unconstrained motion. These properties make PACs and related polycations ideal for drug delivery when the drug needs to reach specific sites in the
10 DNA. In short, polyamine-containing conjugates can act as recognition elements for the polyamine uptake apparatus and may also enhance DNA targeting via their electrostatic PAC-DNA interactions. These properties lay the foundation for a “value-added” vectoring system as indicated in Figure 3. In addition to the demonstrated compounds, by attaching a polyamine architecture (as example, the 4,4 triamine) onto a current drug
15 scaffold (e.g., doxorubicin), the potency and selectivity of the drug may be increased (14, Figure 4). Alternatively, a tetraamine scaffold could also provide enhanced abilities, (15, Figure 4).

This invention identifies efficient polyamine vector architectures required to harness the polyamine transporter. This, in turn, led to new drugs and drug delivery
20 systems, which target rapidly dividing cancer cells over normal resting cells (e.g., melanoma cells over melanocytes, see Table 3). This is a discovery of significant therapeutic value in the fight against cancer. Moreover, this strategy has identified targeted antineoplastic agents, which are non-antibody based, as well as structural

elements, which can be attached to other drugs to assist their entry into cells expressing a polyamine transporter.

The following examples are provided for the purpose of illustration and not limitation.

5

Examples

Synthesis: As shown in Figure 2A, Scheme 1, the reductive amination of **1** to **4** was achieved in two steps via *in situ* generation of the imine **3**. A homologous series of imines (**3a~d**) were prepared from **1** and different alcohols. Each imine was then reduced to its respective amine **4** with NaBH₄ in good yield without purification. Solvent removal
10 by rotary evaporation at 40-50°C facilitated imine formation and provided satisfactory yields of the 2° amines, **4a-d**, (68~81%) after the two-step process. The 2° amines **4a-d** were *N*-protected to form **5** using excess di-*t*-butyl dicarbonate, Boc₂O. Interestingly, compound **5b**, which contained three methylene units, was unstable, even when it was stored at low temperature (0 ~ 5°C) under nitrogen. This finding is in direct contrast to
15 **5a**, **5c** and **5d**, which were stable at room temperature.

In the seemingly routine tosylation step, shown in Scheme 2, we were unable to obtain the desired compound **6a** from the *N*-Boc protected **5a**. In addition, the impurities generated during the formation of **5b** greatly affected the tosylation reaction. Nevertheless, tosylate **6b** was isolated in lower yield (51%), but was unstable to
20 prolonged storage. In contrast, tosylates **6c** and **6d** were prepared in higher yields (88%) and were relatively stable. However, their respective colors and ¹H NMR spectra slowly changed during prolonged storage in the refrigerator. Therefore, the tosylates **6** were best generated and used as soon as possible. Alternatively, methanesulfonyl chloride (i.e.,

mesyl chloride) can be used in lieu of tosyl chloride to activate the alcohol subunit. The advantage to using mesyl chloride is that it is readily removed by a 1N NaOH washing step. The yields using either sulfonylchloride agent are comparable. However, the isolation of synthetic intermediates, where OTs = O-mesyl instead of O-tosyl in Schemes 2-4 and Scheme 6, is more efficient and conducive to scale-up processes.

As shown in Scheme 2, the tosylated compounds (**6b~d**) were reacted with excess putrescine or 1,3-diaminopropane to form six ¹N-Boc protected triamines **7b-g**. These triamines could be cleanly isolated, but were again unstable to prolonged storage at low temperature. Therefore, they were consumed in the next step immediately after purification. The N-Boc groups of **7** were removed by 4N HCl, and the six triamine compounds **8b-g** were formed in good yield. Impurities in **8** were removed by washing the solids with absolute ethanol.

After repeated syntheses, it was found that **5** and **6** could be used directly in subsequent steps to provide satisfactory yields and purities of the target compounds **8**. Therefore, as long as adducts **4** and **7** were pure, one could avoid column chromatography on the other intermediates, **5** and **6**.

As shown in Scheme 3, derivatives **4a-d** were previously converted to their respective *N,O*-bis-tosylates **9a-d**. Displacement of the terminal tosylate by butanediamine provided the series, **10a-d** in good yield. Derivatives **10a-d** represent triamine systems containing two large aromatic motifs and has one of the terminal amines sequestered as a sulfonamide.

As shown in Scheme 4, tetraamine derivatives **13a-i** were synthesized via intermediates **6**, **11**, and **12** using similar methods as shown for **8b-g** in Schemes 1 and 2

(Figure 2A-2B). How these structural perturbations influence the cytotoxicity of the bioconjugate were evaluated via IC_{50} and K_i determinations are listed in Tables 1 & 2.

Figure 5 (Scheme 5) provides an example of a synthetic method to attach the polyamine vector onto an existing chemotherapeutic agent such as doxorubicin, **21**. The use of an antineoplastic agent conjugated to the appropriate polyamine should provide enhanced antineoplastic activity. The synthetic methodology led to the synthesis of the N-Boc protected polyamine-doxorubicin conjugate **23** from which one obtains the doxorubicin-4,4-triamine conjugate, **14**. Coupling an appropriate polyamine to other existing chemotherapeutic agents (e.g., mitoxantrone, anthramycin, camptothecin, vincristine or *cis* platin) should also provide an enhanced chemotherapeutic agent.

As shown in Figure 6A (Scheme 6), the aryl unit (Ar) was varied to include a variety of common arylalkyl units ($ArCH_2$). Using synthetic methods already described in Scheme 2, compounds **26a-d** were reacted with diamines to form triamines **27a,b,d** and **8e**, which contain $R = arylalkyl$. Alternatively, they could be reacted with aminoalcohols, tosylated and reacted with diamines to form the tetraamine analogues similar to that outlined in Figure 2C (Scheme 4) for the synthesis of **13**.

Internal modifications were also made to the aliphatic spacer unit connecting the nitrogens. For example as shown in Figure 6B (Scheme 7), a hydroxy unit (**30**) or a cycloalkyl spacer unit (**31**) were inserted synthetically using a similar reaction of a diamine reacting with a tosylate (similar to how compounds **8** were made in Scheme 2).

Overall, the synthetic method is modular and allows for a variety of structural alterations to be introduced into the general architecture.

Biological Evaluation. Three cell lines were chosen for bioassay. L1210 (mouse leukemia) cells were selected for comparison with the published IC_{50} and K_i values determined for a variety of polyamine substrates. In this regard both K_i and IC_{50} values were measured in this line for comparison purposes. Chinese hamster ovary (CHO) cells were chosen along with a mutant cell line (CHO-MG) in order to comment on polyamine transporter affinity and cell selectivity.. The CHO-MG cell line is polyamine-transport deficient and was isolated after chronic selection for growth resistance to methylglyoxalbis (guanyldiazotization). For the purposes of this study, the CHO-MG cell line represents cells with limited polyamine transport activity. In contrast, the parent CHO cell line illustrates cell types with active polyamine transport. Comparison of efficacy in these two lines provided an important screen to detect conjugate delivery via the polyamine transporter, PAT. For example, a conjugate with high utilization of the transporter would be very toxic to CHO cells, but less so to CHO-MG cells. Therefore, IC_{50} determination in these two CHO lines provided a relative ranking of delivery via the PAT. In short, highly selective, vectored conjugates would give high (CHO-MG/CHO) IC_{50} ratios.

It is a well-known practice in pharmaceutical science to use pharmaceutically acceptable acid salts of amine derivatives to facilitate their long storage and dosing as aqueous solutions. The examples listed in this invention are comprised of a polyamine salt derived from a pharmaceutically acceptable acid (e.g., HCl) with or without the use of a pharmaceutically acceptable carrier (e.g., water). Such salts can be derived from either inorganic or organic acids, including for example hydrochloric, hydrobromic, acetic, citric, fumaric, maleic, benzenesulfonic, and ascorbic acids. The pharmaceutical

compositions obtained by the combination of the carrier and the polyamine salt will generally be used in a dosage necessary to elicit the desired biological effect. This includes its use in an antineoplastic effective amount or in a lesser amount when used in combination with other biologically active agents.

5 **Table 1.** Biological Evaluation of triamines **8b-g**, *N*-tosyl derivatives **10a-d**, tetraamines **13a-i**, homologues **27**, and controls **30-35** in L1210 cells.

Compd (tether)	L1210 IC ₅₀ in μ M	L1210+DFMO IC ₅₀ in μ M	L1210/(L1210+DFMO) IC ₅₀ ratio	K _i values (μ M) L1210 cells
8b (3,3)	1.8 (\pm 0.4)	1.4 (\pm 0.3)	1.3	33.4 (\pm 2.6)
8c (3,4)	0.7 (\pm 0.3)	0.3 (\pm 0.1)	2.3	2.5 (\pm 0.3)
8d (4,3)	0.4 (\pm 0.1)	0.2 (\pm 0.02)	2	6.2 (\pm 0.6)
8e (4,4)	0.3 (\pm 0.04)	0.15 (\pm 0.1)	2	1.8 (\pm 0.1)
8f (5,3)	1.3 (\pm 0.1)	0.7 (\pm 0.1)	1.9	5.0 (\pm 0.6)
8g (5,4)	0.4 (\pm 0.1)	0.3 (\pm 0.1)	1.3	1.7 (\pm 0.2)
10a (2,4)	3.3 (\pm 0.2)	3.9 (\pm 0.9)	0.9	ND
10b (3,4)	6.3 (\pm 0.5)	7.7 (\pm 1.1)	0.8	ND
10c (4,4)	7.4 (\pm 1.0)	8.1 (\pm 1.6)	0.9	ND
10d (5,4)	6.2 (\pm 0.3)	6.9 (\pm 0.8)	0.9	ND
13a (3,3,4)	21.8 (\pm 3.2)	21.9 (\pm 4.3)	1	0.107 (\pm 0.013)
13b (3,4,3)	19.5 (\pm 2.8)	31.9 (\pm 1.9)	0.6	0.202 (\pm 0.008)
13c (3,4,4)	9.8 (\pm 1.7)	5.1 (\pm 0.6)	1.9	0.079 (\pm 0.009)

13d (3,5,4)	10.7 (± 2.4)	7.2 (± 0.1)	1.5	0.090 (± 0.006)
13e (4,4,3)	4.3 (± 0.6)	0.6 (± 0.2)	7.2	0.074 (± 0.005)
13f (4,4,4)	7.5 (± 0.3)	1.6 (± 0.3)	4.7	0.051 (± 0.006)
13g (5,4,3)	6.4 (± 1.0)	2.1 (± 0.7)	3	0.099 (± 0.008)
13h (5,4,4)	7.2 (± 0.6)	3.8 (± 0.1)	1.9	0.065 (± 0.005)
13i (5,5,4)	7.1 (± 1.2)	4.3 (± 0.9)	1.7	0.064 (± 0.005)
27a:benzyl (4,4)	36.3 (± 8.4)	421 (± 27.1)	0.1	4.5 (± 0.8)
27b:naphthyl (4,4)	0.50 (± 0.03)	0.43(± 0.02)	1.1	3.8 (± 0.5)
27d:pyrenyl (4,4)	0.40 (± 0.02)	0.36(± 0.06)	1.1	2.9 (± 0.3)
30:Ant- hydroxyamino	1.50 (± 0.08)	2.30(± 0.29)	0.7	12.5 (± 1.2)
31:Ant- (cyclohexyl)	1.00 (± 0.16)	0.7 (± 0.1)	1.4	3.8 (± 0.9)
32:Ant- (octylene)	3.00 (± 0.07)	4.60(± 0.12)	0.7	13.3 (± 1.5)
33:Ant- (diethoxy)	11.30 (± 0.37)	17.0(± 0.61)	0.7	90.0 (± 4.6)
34:Ant- butanediamine	6.30 (± 0.26)	9.78(± 0.42)	0.7	32.2 (± 4.3)
35:Ant(N- butyl)	14.6 (± 0.1)	21.9 (± 3.6)	0.7	62.3 (± 4.2)

As shown in Table 1, L1210 cells which were pretreated with DFMO were more susceptible to the polyamine conjugates, which use the polyamine transporter (**8b-8g**). In contrast, DFMO pretreatment slightly reduced the potency of systems, which do not use the transporter (**10a-10d**). These conclusions were reached from the following two trends: a) the IC_{50} values were lower for the triamine series (**8b-8g**) which use the transporter upon DFMO pretreatment and the IC_{50} values were higher for the triamine series which have limited use of the transporter (**10a-d**) upon DFMO pretreatment. As expected, the tetraamine systems **13a-i** were also effective in killing DFMO-pretreated cells. The homologous series **27b**, **8e** and **27d** (in Figure 6A, Scheme 6) revealed the structural tolerance of the polyamine transporter to import *N*-alkylaryl units of varying size and hydrophobicity. Research has shown that the compounds of the invention, mono-substituted N^1 -alkylaryl triamines, have enhanced selectivity and cytotoxicity. The fact that **8e** and cyclohexyl derivative **31** also had similar properties suggests that further alterations of the polyamine chain can be accommodated by the transporter. As expected, the control compounds **32-35** (Figure 6) were all less cytotoxic than the vectored triamine systems, **8**.

The K_i values in Table 1 reflect the affinity of the polyamine derivative for the transport apparatus on the cell surface. The lower the K_i value, the higher the affinity. Triamine-anthracene conjugates (which have a 4,4 triamine **8e** and 5,4 triamine **8g** sequence) were preferred and demonstrated the highest affinity for the polyamine transporter of the triamines tested. The tetraamines **13** had the lowest K_i values and reflected their superior affinity for the polyamine transporter. However, their IC_{50} values were typically higher than the corresponding triamine systems, which means they are less

cytotoxic. This clearly illustrates that high affinity for the polyamine transporter does not always translate into higher cytotoxicity.

Table 2. Biological Evaluation of triamines **8b-g**, *N*-tosyl derivatives **10a-d** and tetraamines **13a-i**, homologues **27**, and controls **30-35**, in the CHO and CHO-MG cell lines.

Compd (tether)	CHO-MG IC ₅₀ in μ M	CHO IC ₅₀ in μ M	CHO-MG/CHO IC ₅₀ ratio
8b (3,3)	3.4 (\pm 0.5)	1.9 (\pm 0.4)	1.8
8c (3,4)	8.8 (\pm 1.2)	2.5 (\pm 0.7)	3.5
8d (4,3)	9.5 (\pm 1.1)	0.4 (\pm 0.1)	24
8e (4,4)	66.7 (\pm 4.1)	0.45 (\pm 0.1)	148
8f (5,3)	10.1 (\pm 1.2)	4.1 (\pm 0.5)	2.5
8g (5,4)	57.3 (\pm 2.9)	1.5 (\pm 0.1)	38
10a (2,4)	7.1 (\pm 0.4)	5.1 (\pm 0.6)	1.4
10b (3,4)	11.1 (\pm 1.3)	10.2 (\pm 0.9)	1.1
10c (4,4)	10.6 (\pm 1.9)	10.4 (\pm 1.6)	1.0
10d (5,4)	7.8 (\pm 1.9)	7.1 (\pm 0.8)	1.1
13a (3,3,4)	41.5 (\pm 3.5)	44 (\pm 0.0)	0.9
13b (3,4,3)	75.7 (\pm 7.3)	59.7 (\pm 6.5)	1.3
13c (3,4,4)	52.8 (\pm 2.6)	31.2 (\pm 7.3)	1.7
13d (3,5,4)	41.7 (\pm 0.2)	35 (\pm 1.3)	1.2
13e (4,4,3)	2.8 (\pm 0.4)	4 (\pm 1.4)	0.7

13f (4,4,4)	33.2 (± 1.7)	10.6 (± 0.0)	3.1
13g (5,4,3)	33.5 (± 3.5)	18 (± 3.5)	1.9
13h (5,4,4)	30.8 (± 0.4)	9.9 (± 1.6)	3.1
13i (5,5,4)	5.7 (± 1.6)	4 (± 0.8)	1.4
27a :benzyl (4,4)	>1000	>1000	NA
27c :naphthyl (4,4)	>100	0.6 (± 0.2)	>164
27d :pyrenyl (4,4)	15.5 (± 2.4)	0.46 (± 0.05)	34
30 :Ant-(4,3- hydroxyamino)	9.5 (± 0.8)	9.1 (± 0.4)	1
31 :Ant- (cyclohexyl)	17.4 (± 2.8)	2.5 (± 0.5)	7
32 :Ant- (octylene)	4.9 (± 0.1)	4.9 (± 0.2)	1
33 :Ant- (diethoxy)	15.9 (± 1.5)	12.6 (± 0.6)	1.3
34 : Ant- diamine	7.6 (± 0.4)	7.7 (± 0.5)	1
35 : Ant (N-butyl)	11.2 (± 2.3)	10.5 (± 2.0)	1.1

As shown in Table 2, biological evaluation of triamines **8b-g** and **10a-d** in CHO cells revealed that the 4,4-triamine **8e** displays a nearly 150-fold preference for the CHO line containing PAT over CHO-MG, while the 3,3-triamine analogue **8b** preferred this line by only 1.8 fold. Note: the entire tetraamine series **13** had, at best, only a 3 fold

preference or lower. The naphthyl derivative **27b** also had an excellent selectivity profile with >164 fold preference in killing cells with an active polyamine transport system. These findings demonstrate that triamine conjugates (particularly **8e** and **27b**, which both used the 4,4 triamine vector) make preferred cell targeting motifs. The effectiveness of the triamines is also reflected in the L1210 IC₅₀ results, wherein the 4,4-triamine **8e** and 5,4-triamine **8g** showed a greater sensitivity to DFMO treatment. Therefore, the triamine systems seem to give consistent data in all three cell lines.

Thus, the 4,4-triamine architecture represents a preferred vectoring system, which upon attachment to a toxic agent imparts high cell selectivity and low IC₅₀ values in the CHO and L1210 cell lines (Tables 1 and 2). In short, one can selectively deliver “large” toxic agents to tumor cells with highly active polyamine transporters by using the proper polyamine system for cell targeting.

An example of selectively targeting cancer cells (melanoma) with this strategy is illustrated in Table 3.

Table 3. Cell selectivity profile for **8e** and the control *N*-butyl derivative **35**.

Cell Type	Time	Ant-4,4-triamine 8e IC ₅₀ (μM)	Ant- <i>N</i> -butylamine 35 IC ₅₀ (μM)
B16 (melanoma)	24 h	1.93 (± 0.11)	19.25 (± 2.76)
B16 (melanoma)	48 h	1.10 (± 0.07)	21.31 (± 2.18)
B16 (melanoma)	72 h	0.62 (± 0.03)	20.39 (± 1.81)
Mel-A(normal melanocyte)	24 h	16.47 (± 1.95)	44.30 (± 9.14)
Mel-A(normal melanocyte)	48 h	8.27 (± 0.95)	32.80 (± 4.64)
Mel-A(normal melanocyte)	72 h	6.49 (± 1.15)	15.00 (± 3.73)

Many cancer cell lines are known to have high PAT activity, and should be susceptible to this vectored chemotherapeutic strategy. As shown in Table 3, the

triamine-vectored **8e** analogue was 7.5-10.5 times more cytotoxic to melanoma B16 cells than the normal melanocytes, Mel-A (e.g. $6.49/0.62 = 10.5$). The Ant-*N*-butyl control **35** (Figure 7), which has been shown to not use the PAT, was only 0.7-2.3 fold more potent. Since the respective cell doubling times are different, IC_{50} ratios (**35/8e**) for each cell type were also compared. This alternative interpretation revealed that the presence of the triamine vector in **8e** resulted in 10-32 fold higher cytotoxicity in B-16 cells, which have high PAT activity, than control **35** (e.g., 24h: $19.25/1.93 = 10$). In contrast, the triamine vector in **8e** resulted in only 2-4 fold higher cytotoxicity in Mel-A cells than **35** (e.g. 24 h: $44.3/16.47 = 2.7$). These results further support the proposed polyamine vector strategy as a viable means to target cancer cells over their healthy counterparts.

With respect to the tetraamine systems, **13**, the 4,4,4-tetraamine **13f** and the 5,4,4-tetraamine **13h** show enhanced selectivity in killing cells with active polyamine transporters (Table 2). The three-fold enhancement in cytotoxicity, while not as remarkable as the related triamine systems, distinguished these two tetraamines as the best of the tetraamine series studied.

Experimental Section

Materials. Silica gel (32-63 μ m) was purchased from Scientific Adsorbents Incorporated. Chemical reagents were purchased either from the ACROS Chemical Co. or the Sigma Chemical Co. and used without further purification. All solvents were distilled prior to use. 1H NMR and ^{13}C NMR spectra were recorded at 300 and 75 MHz, respectively. TLC solvent systems are based on volume % and NH_4OH refers to concentrated aqueous NH_4OH . All final compounds listed in the Tables had satisfactory elemental analyses (a proof of purity).

General Procedure for the Synthesis of *N*-(Anthracen-9-ylmethyl) amino alcohols 4.

To a stirred solution of amino alcohol (12 mmol) in 25% MeOH /CH₂Cl₂ (10 mL) was added a solution of 9-anthraldehyde (10 mmol) in 25% MeOH /CH₂Cl₂ (10 mL) under N₂. The mixture was stirred at room temperature overnight until the imine formation was complete (monitored by TLC). The solvent was evaporated under vacuum to give the crude imine as a bright green solid, which was used for reduction without further purification.

NaBH₄ (30 mmol) was added in small portions to the solution of imine 1:1 CH₃OH :CH₂Cl₂ (20 mL) at 0 °C . The mixture was stirred at room temperature overnight, then concentrated under vacuum. The residue was dissolved in CH₂Cl₂ (50 mL), and washed three times with aq. Na₂CO₃ (pH 10, 50 mL). The organic layer was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel.

2-[(Anthracen-9-ylmethyl)-amino] ethanol, 4a. Bright yellow solid; mp 116-118 °C; yield 77%; R_f=0.48, methanol/chloroform, 1:9; ¹H NMR (300 MHz, CDCl₃): δ 8.40 (s, 1H), 8.28 (d, 2H), 8.00 (d, 2H), 7.48 (m, 4H), 4.68 (s, 2H), 3.64 (t, 2H), 3.00 (t, 2H), 2.1 (br s, 2H); ¹³C NMR: δ 131.75, 131.44, 130.45, 129.47, 127.63, 126.48, 125.23, 124.18, 61.12, 51.65, 45.41; Anal. Calcd. for C₁₇H₁₇NO: C, 81.24; H, 6.82; N, 5.57; found: C, 81.28; H, 6.83; N, 5.57; HRMS (FAB) m/z calcd. for C₁₇H₁₈NO (M+H)⁺: 252.1388; found: 252.1381.

3-[(Anthracen-9-ylmethyl)-amino]-propan-1-ol, 4b. Bright yellow solid; mp 82-83 °C; yield 80%; R_f = 0.51, methanol/chloroform, 1:9; ¹H NMR (300 MHz, CDCl₃): δ 8.40 (s, 1H), 8.28 (d, 2H), 8.00 (d, 2H), 7.46 (m, 4H), 4.70 (s, 2H), 3.80 (t, 2H), 3.10 (t, 2H), 2.90

(br s, 2H), 1.78 (m, 2H); ^{13}C NMR: δ 131.72, 130.98, 130.48, 129.48, 127.72, 126.56, 125.25, 124.06, 64.65, 50.83, 46.02, 31.14. Anal. Calcd. for $\text{C}_{18}\text{H}_{19}\text{NO}$: C, 81.48; H, 7.22; N, 5.28. Found: C, 81.20; H, 7.20; N, 5.28. HRMS(FAB) m/z calcd. for $\text{C}_{18}\text{H}_{20}\text{NO}(\text{M}+\text{H})^+$: 266.1545; found: 266.1526.

- 5 **4--[(Anthracen-9-ylmethyl)-amino]-butan-1-ol, 4c.** Bright yellow solid; mp 87-88 °C ; yield 81%; R_f = 0.51, methanol/chloroform, 1:9; ^1H NMR (300 MHz, CDCl_3) δ 8.40 (s, 1H), 8.26 (d, 2H), 8.00 (d, 2H), 7.49 (m, 4H), 4.68 (s, 2H), 3.50 (t, 2H), 2.90 (t, 2H), 1.65 (br s, 4H); ^{13}C NMR: δ 131.74, 130.73, 130.48, 129.51, 127.82, 126.64, 125.30, 123.96, 62.89, 50.58, 45.72, 32.72, 29.13. Anal. Calcd. for $\text{C}_{19}\text{H}_{21}\text{NO}$: C, 81.68; H, 7.58; N, 5.01. found: C, 81.63; H, 7.65; N, 5.10. HRMS (FAB) m/z calcd. for: $\text{C}_{19}\text{H}_{22}\text{NO}(\text{M}+\text{H})^+$: 280.1701; found: 280.1679.

- 15 **5--[(Anthracen-9-ylmethyl)-amino]-pentan-1-ol, 4d.** Bright yellow solid; mp 76-77 °C; yield 68%; R_f = 0.26, methanol/chloroform (5:95); ^1H NMR (CDCl_3): δ 8.38 (s, 1H), 8.27 (d, 2H), 7.98 (d, 2H), 7.45 (m, 4H), 4.65 (s, 2H), 3.50 (t, 2H), 2.82(t, 2H), 1.78 (br s, 2H), 1.50 (m, 4H), 1.40 (m, 2H); ^{13}C NMR (CDCl_3): δ 131.89, 131.77, 130.48, 129.44, 127.46, 126.40, 125.19, 124.29, 62.68, 50.60, 45.96, 32.68, 29.91, 23.67. Anal. Calcd. for $\text{C}_{20}\text{H}_{23}\text{NO}$: C, 81.87; H, 7.91; N, 4.77. found: C, 81.89; H, 7.99; N, 4.86. HRMS (FAB) m/z calcd. for $\text{C}_{20}\text{H}_{24}\text{NO}(\text{M}+\text{H})^+$: 294.1858; found: 294.1835.

- 20 **The General Procedure for the N-Boc protection of N-(Anthracen-9-ylmethyl)-amino alcohols 4 to give 5.** The solution of *N*-(anthracen-9-ylmethyl)-amino alcohol (5 mmol) in 20 mL of pyridine-methanol (1:5 v/v) was stirred at 0 °C for 10 min. A solution of di-tert -butyl dicarbonate (7.5 mmol) in methanol (5 mL) was added dropwise over ten minutes. The temperature was allowed to rise to room temperature and the reaction was

stirred overnight. The mixture was evaporated to dryness under reduced pressure. The residue was dissolved in methylene chloride, and washed with deionized water several times. The organic layer was separated, dried over anhydrous Na₂SO₄, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica
5 gel.

Anthracen-9-ylmethyl-(2-hydroxy-ethyl) carbamic acid *tert*-butyl ester, 5a. Pale yellow solid; mp 131-132 °C; yield 84%; R_f = 0.21 (acetone/hexane 1:4); ¹H NMR (CDCl₃): δ 8.43 (s, 1H), 8.38 (br s, 2H), 8.01 (d, 2H), 7.43 (m, 4H), 5.50 (br s, 2H), 3.30 (t, 2H), 3.00 (br s, 3H, including -OH), 1.52 (br s, 9H). Anal. Calcd. for C₂₂H₂₅NO₃: C, 75.19; H, 7.17, N, 3.98. found. C, 75.02, H, 7.13, N, 3.98.
10

Anthracen-9-ylmethyl-(3-hydroxy-propyl) carbamic acid *tert*-butyl ester, 5b. Unstable pale yellow solid; yield 90%, R_f = 0.23 (acetone/hexane 1:4); ¹H NMR (CDCl₃): δ 8.42 (s, 1H), 8.36 (d, 2H), 8.02 (d, 2H), 7.52 (m, 4H), 5.50 (br s, 2H), 3.25 (br s, 2H), 3.10 (br s, 2H), 1.62 (m, 11H). HRMS (FAB): calcd. for C₂₃H₂₈NO₃ (M+H)⁺: 366.2069; Found: 366.2067.
15

Anthracen-9-ylmethyl-(4-hydroxy-butyl) carbamic acid *tert*-butyl ester, 5c. Pale yellow solid; mp 113-114 °C; yield 88%; R_f = 0.13(acetone/hexane 12:88); ¹H NMR (CDCl₃): δ 8.42 (s, 1H), 8.39 (d, 2H), 8.01 (d, 2H), 7.52 (m, 4H), 5.50 (br s, 2H), 3.25 (t, 2H), 2.80 (br s, 2H), 1.60 (br s, 9H), 1.20 (m, 4H). ¹³C NMR: δ 155.88, 131.52, 131.48, 129.45, 128.99, 126.53, 125.22, 124.32, 80.08, 62.35, 44.54, 41.28, 30.05, 28.94
20 (3C), 24.99. Anal. Calcd. for C₂₄H₂₉NO₃: C, 75.96; H, 7.70; N, 3.69. found: C, 76.04; H, 7.64; N, 3.68. HRMS (FAB): calcd. for C₂₄H₂₉NO₃Na (M+Na)⁺: 402.2045; found: 402.2070.

Anthracen-9-ylmethyl-(5-hydroxy-pentyl) carbamic acid tert-butyl ester, 5d. Pale yellow solid; mp 125-126 °C; yield 84%; R_f = 0.28 (acetone/hexane 1:4); ^1H NMR (CDCl_3): δ 8.44 (s, 1H), 8.41 (d, 2H), 8.05 (d, 2H), 7.55 (m, 4H), 5.56 (br s, 2H), 3.38 (t, 2H), 2.82 (br s, 2H), 1.64 (br s, 9H), 1.25 (m, 4H), 0.98 (m, 2H); ^{13}C NMR: δ 155.97, 131.56, 131.53, 129.43, 128.34, 126.51, 125.23, 124.43, 79.94, 62.85, 44.84, 41.41, 32.33, 28.96(3C), 28.43, 23.14. Anal. Calcd. for $\text{C}_{25}\text{H}_{31}\text{NO}_3$: C, 76.30; H, 7.94; N, 3.56. found: C, 76.29; H, 7.96; N, 3.67. HRMS (FAB): calcd. for $\text{C}_{25}\text{H}_{32}\text{NO}_3 (\text{M}+\text{H})^+$: 394.2382; found: 394.2385.

General Procedure for the tosylation of N-Boc protected (anthracen-9-ylmethyl)-amino alcohols 4 to give 9. A solution of the N-Boc protected (anthracen-9-ylmethyl)-amino alcohol (5 mmol) in 20 mL dry pyridine was stirred at 0 °C for 10 min. *p*-Toluenesulfonyl chloride (TsCl, 7.5 mmol) was added in small portions over 30 min. The mixture was stirred for an additional hour and the reaction flask was placed in a refrigerator (0-5 °C) overnight. The mixture was poured into 200 mL of ice-water, and a hemi-solid (or viscous liquid) typically precipitated (or separated). After decanting off the upper layer, the residue was dissolved in methylene chloride and washed several times with deionized water. The organic layer was separated, dried over anhydrous Na_2SO_4 , filtered and concentrated under vacuum. The residue was purified by flash chromatography on silica gel.

Toluene-4-sulfonic acid 3-(anthracen-9-ylmethyl-*tert*-butoxycarbonyl-amino)-propyl ester, 9b. Unstable bright yellow viscous liquid, yield 51%; R_f = 0.21 (acetone/hexane 1:4). ^1H NMR (CDCl_3): δ 8.42 (s, 1H), 8.39 (d, 2H), 8.05 (d, 2H), 7.58 (m, 6H), 7.21 (d,

2H), 5.46 (s, 2H), 3.63 (t, 2H), 2.82 (t, 2H), 2.41 (s, 3H), 1.64 (br s, 11H). HRMS (FAB):
calcd. for $C_{25}H_{26}NO_3S$ ($M+2H-Boc$)⁺: 420.1633 ; found: 420.1642.

Toluene-4-sulfonic acid 4-(anthracen-9-ylmethyl-*tert*-butoxycarbonyl-amino)-butyl

ester, 9c. Pale yellow viscous liquid; yield 88%; R_f = 0.38, acetone/hexane 1:3; ¹H NMR
(CDCl₃): δ 8.42 (s, 1H), 8.37 (d, 2H), 8.00 (d, 2H), 7.62 (d, 2H), 7.48 (m, 4H), 7.22 (d,
2H), 5.46 (s, 2H), 3.63 (t, 2H), 2.77 (br s, 2H), 2.40 (s, 3H), 1.48 (br s, 9H), 1.20 (br s,
4H); Anal. Calcd. for $C_{31}H_{35}NO_5S \cdot 0.5H_2O$: C, 68.61; H, 6.69; N, 2.58. found: C, 68.74;
H, 6.57; N, 2.55. HRMS : calcd. for $C_{31}H_{35}NO_5S$ M⁺: 533.2236; found: 533.2236.

Toluene-4-sulfonic acid 5-(anthracen-9-ylmethyl-*tert*-butoxycarbonyl-amino)-pentyl

ester, 9d. Pale yellow viscous liquid; yield 88%; R_f = 0.25, acetone/hexane 1:4; ¹H NMR
(CDCl₃): δ 8.42 (s, 1H), 8.36 (d, 2H), 8.00 (d, 2H), 7.63 (d, 2H), 7.46 (m, 4H), 7.22 (d,
2H), 5.47 (s, 2H), 3.73 (br s, 2H), 2.76 (br s, 2H), 2.40 (s, 3H), 1.48 (br s, 9H), 1.24 (br
s, 2H), 1.20 (br s, 2H), 0.93 (br s, 2H); Anal. Calcd. for $C_{32}H_{37}NO_5S \cdot 0.5H_2O$: C, 69.04;
H, 6.88; N, 2.52. found: C, 69.15; H, 6.75; N, 2.57. HRMS (FAB): calcd. for
 $C_{32}H_{38}NO_5S$ ($M+H$)⁺: 548.2471; found: 548.2501.

General Procedure for the preparation of the N¹- Boc protected- N¹-(Anthracen-9-ylmethyl)-triamines, 7. The tosylated products **6** (1 mmol) and 1,4-diaminobutane or 1,3-diaminepropane (10 mmol) were dissolved in acetonitrile (10 mL), then stirred at 75 °C under N₂ overnight. After checking for the disappearance of the tosylate by TLC, the solution was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (20 mL) and washed three times with saturated aqueous sodium carbonate. The organic layer was separated, dried over anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by flash chromatography on silica gel. The

purified products were used immediately for next step (BOC deprotection). The isolated yields ranged between 59-75%.

General Procedure for the preparation of the N^1 - (Anthracen-9-ylmethyl)-triamines,

8. The N^1 - Boc protected- N^1 -(anthracen-9-ylmethyl)-triamine **7** (0.5 mmol) was dissolved in ethanol (5 mL), and stirred at 0 °C for 10 min. 4 N aq. HCl (8 mL) was added dropwise at 0 °C. The mixture was stirred at room temperature overnight. The solution was then concentrated under reduced pressure (while maintaining the water bath on the rotary evaporator below 60 °C) and a bright yellow solid precipitated. The solids were washed several times with absolute ethanol and provided the pure target compounds. The ^1H NMR spectra of polyamine conjugates were measured in 0.5 mL DMSO- d_6 and 3 drops of D_2O . The use of DMSO- d_6 / D_2O mixtures resulted in better spectral resolution (compared to using pure D_2O as solvent). The ^{13}C NMR spectra of the triamines were measured in D_2O to avoid the interference of DMSO carbon signals. The listed amines are all in their HCl salt form.

N -(3-Amino-propyl)- N -anthracen-9-ylmethyl-propane-1,3-diamine,

trihydrochloride 8b. Bright yellow solid, yield 98%. ^1H NMR (DMSO- d_6 + D_2O): δ 8.80 (s, 1H), 8.40 (d, 2H), 8.22 (d, 2H), 7.75 (t, 2H), 7.62 (t, 2H), 5.23 (s, 2H), 3.40 (t, 2H), 3.05 (m, 4H), 2.96 (t, 2H), 2.18 (m, 2H), 2.00 (m, 2H); ^{13}C NMR: δ 130.63, 130.45, 130.06, 129.47, 127.72, 125.50, 122.48, 120.06, 44.91, 44.82, 44.75, 43.08, 36.74, 23.98, 22.93. Anal. Calcd. for $\text{C}_{21}\text{H}_{30}\text{Cl}_3\text{N}_3$: C, 58.54; H, 7.02; N, 9.75. found: C, 58.27; H, 6.90; N, 9.69. HRMS (FAB): calcd. for $\text{C}_{21}\text{H}_{30}\text{Cl}_2\text{N}_3$ ($\text{M}+\text{H}-\text{HCl}$) $^+$: 394.1817; found: 394.1806.

***N'*-[3-[(Anthracen-9-ylmethyl)-amino]-propyl]-butane-1,4-diamine,**

trihydrochloride 8c. Bright yellow solid, yield 98%. ¹H NMR (DMSO-d₆+D₂O): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.72 (t, 2H), 7.62 (t, 2H), 5.23 (s, 2H), 3.42 (t, 2H), 3.05 (t, 2H), 2.98 (t, 2H), 2.82 (t, 2H), 2.20 (br s, 2H), 1.71 (br s, 4H). ¹³C NMR (D₂O):
5 δ 130.57, 130.41, 130.01, 129.45, 127.71, 125.48, 122.46, 119.98, 47.27, 44.76, 44.66, 43.01, 39.03, 24.16, 23.00, 22.92. Anal. Calcd. for C₂₂H₃₂Cl₃N₃ 0.6 H₂O: C, 57.99; H, 7.34; N, 9.22. found: C, 58.00; H, 7.36; N, 9.20. HRMS (FAB): calcd. for C₂₂H₃₂Cl₂N₃ (M+H-HCl)⁺: 408.1973; found: 408.1950.

***N*-(3-Amino-propyl)-*N*-anthracen-9-ylmethyl-butane-1,4-diamine, trihydrochloride**
10 **8d.** Bright yellow solid, yield 95%. ¹H NMR (DMSO-d₆+ D₂O): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.73 (t, 2H), 7.64 (t, 2H), 5.23 (s, 2H), 3.30 (t, 2H), 2.99 (m, 6H), 2.00 (m, 2H), 1.80 (m, 4H). ¹³C NMR(D₂O): δ 130.65, 130.37, 130.04, 129.48, 127.70, 125.51, 122.49, 120.30, 47.18(2C), 44.78, 42.79, 36.81, 24.04, 23.14, 22.99. Anal. Calcd. for C₂₂H₃₂Cl₃N₃ : C, 59.40; H, 7.25; N, 9.45. found: C, 59.48; H, 7.07; N, 9.30. HRMS
15 (FAB): calcd. for C₂₂H₃₂Cl₂N₃ (M+H-HCl)⁺: 408.1973; found: 408.1958.

***N*-(4-Amino-butyl)-*N*-anthracen-9-ylmethyl-butane-1,4-diamine, trihydrochloride**
8e. Bright yellow solid, yield 91%. ¹H NMR (DMSO-d₆ + D₂O): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.70 (t, 2H), 7.61 (t, 2H), 5.23 (s, 2H), 3.30 (t, 2H), 2.93 (m, 4H), 2.82 (t, 2H), 1.78-1.60 (m, 8H). ¹³C NMR (D₂O): δ 130.62, 130.32, 130.01, 129.45, 127.67,
20 125.48, 122.48, 120.37, 47.17, 47.11, 47.00, 42.76, 39.03, 24.19, 23.14, 23.02 (2C). Anal. Calcd. for C₂₃H₃₄Cl₃N₃ 0.8H₂O: C, 58.37; H, 7.58; N, 8.88. found: C, 58.39; H, 7.36; N, 8.76. HRMS (FAB): calcd. for C₂₃H₃₂N₃ (M+H-3HCl)⁺: 350.2590; found: 350.2611.

***N*-(3-Amino-propyl)-*N*-anthracen-9-ylmethyl-pentane-1,5-diamine, trihydrochloride**

8f. Bright yellow solid, yield 86%. ¹H NMR (DMSO-d₆+ D₂O): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.72 (t, 2H), 7.64 (t, 2H), 5.22 (s, 2H), 3.28 (t, 2H), 2.99 (m, 6H), 2.00 (m, 2H), 1.80 (m, 2H), 1.72 (m, 2H), 1.42 (m, 2H); ¹³C NMR (D₂O): δ 130.54, 130.23, 129.91, 129.39, 127.60, 125.42, 122.42, 120.30, 47.62, 47.54, 44.69, 42.56, 36.80, 25.28, 25.22, 24.01, 23.20. Anal. Calcd. for C₂₃H₃₄Cl₃N₃ 0.2H₂O: C, 59.73; H, 7.50; N, 9.09. found: C, 59.71; H, 7.40; N, 9.06. HRMS (FAB): calcd. for C₂₃H₃₄Cl₂N₃ (M+H-HCl)⁺: 422.2130; found: 422.2106.

***N*-(4-Amino-butyl)-*N*-anthracen-9-ylmethyl-pentane-1,5-diamine, trihydrochloride**

8g. Bright yellow solid, yield 88%. ¹H NMR (DMSO-d₆+ D₂O): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.73 (t, 2H), 7.64 (t, 2H), 5.23 (s, 2H), 3.26 (t, 2H), 2.93 (br s, 4H), 2.82 (t, 2H), 2.00 (m, 2H), 1.80 (m, 2H), 1.72 (br s, 4H), 1.42 (m, 2H); ¹³C NMR (D₂O): δ 130.49, 130.21, 129.89, 129.38, 127.61, 125.41, 122.42, 120.23, 47.51, 47.46, 47.07, 42.50, 39.06, 25.29, 25.19, 24.22, 23.20, 23.04. Anal. Calcd. for C₂₄H₃₆Cl₃N₃: C, 60.95; H, 7.67; N, 8.89. found: C, 60.74; H, 7.63; N, 8.79. HRMS (FAB): calcd. for C₂₄H₃₆Cl₂N₃ (M+H-HCl)⁺: 436.2286; found: 436.2289.

General Procedure for the Substitution of the Tosylated Compounds with the amino alcohols or diamines (Preparation of 11 or 13). The tosylated products (compounds 6 or 12) (1 mmol) and ω-amino-α-alcohols (5 mmol) (or diamines when making 13) were dissolved in acetonitrile (10 mL), then stirred at 75 °C under N₂ overnight. After checking the disappearance of tosylated products by TLC, the solution was concentrated under reduced pressure. The residue was dissolved in CH₂Cl₂ (20 mL), washed three times with saturated aqueous sodium carbonate. The organic layer was separated, dried with

anhydrous sodium sulfate, filtered, and concentrated under vacuum. The residue was purified by flash column on silica gel. The purified products **11** were used for next step immediately. During the synthesis of **13**, an intermediate compound (**13x**), which still contained two N-BOC groups was isolated and immediately converted to **13** as described below.

General Procedure for the Amino Group Deprotection (Preparation of 13) The N-

Boc protected N¹-(Anthracen-9-ylmethyl)-tetraamines (**13x**, 0.5 mmol) were dissolved in ethanol (5 mL), and stirred at 0°C for 10 min. 4N HCl (8 mL) then added dropwise at 0°C. The mixture was stirred at room temperature overnight. After that the solution was concentrated under reduced pressure below 60°C, and the bright yellow solid precipitated. The solid was washed with 100% ethanol for several times and to give the pure target compounds **13**.

N¹-{3-[3-(Anthracen-9-ylmethyl)-amino]-propylamino}-butane-1,4-diamine

Tetrahydrochloride (13a) Bright yellow solid; yield 96%; ¹H NMR : δ 8.72 (s, 1H),

8.28 (d, 2H), 8.16 (d, 2H), 7.64 (t, 2H), 7.58 (t, 2H), 5.20 (br s, 2H), 3.38 (br s, 2H), 3.00(m, 8H), 2.84 (br s, 2H), 2.12 (br s, 2H), 2.00 (br s, 2H), 1.62(br s, 4H). ¹³CNMR:

δ130.73, 130.54, 130.17, 129.55, 127.82, 125.59, 122.57, 120.17, 47.38, 44.91(2C),

44.83, 44.70, 43.19, 39.11, 24.25, 23.09, 22.99(2C). Anal. Calcd. for C₂₅H₄₀Cl₄N₄ H₂O :

C, 53.96; H, 7.61; N, 10.07. found: C, 53.89; H, 7.62; N, 10.08. HRMS(FAB): calcd. for

C₂₅H₃₇N₄ (M+H-4HCl)⁺: 393.3013; found: 393.3020

N-(3-Amino-propyl)-N-{3-[(anthracen-9-ylmethyl)-amino]-propyl}-butane-1,4-

diamine Tetrahydrochloride (13b) Bright yellow solid; yield 91%; ¹H NMR : δ 8.73

(s, 1H), 8.28 (d, 2H), 8.17 (d, 2H), 7.64 (t, 2H), 7.58 (t, 2H), 5.18 (s, 2H), 3.30 (t, 2H),

2.96(m, 10H), 2.10 (m, 2H), 1.90 (m, 2H), 1.60(br s, 4H). ^{13}C NMR: δ 130.70, 130.51, 130.13, 129.51, 127.77, 125.55, 122.54, 120.15, 47.25(2C), 44.81(2C), 44.71, 43.12, 36.81, 24.06, 23.05(2C), 22.97. Anal. Calcd. for $\text{C}_{25}\text{H}_{40}\text{Cl}_4\text{N}_4 \cdot 0.4\text{H}_2\text{O}$: C, 55.03; H, 7.54; N, 10.27. found: C, 55.02; H, 7.49; N, 10.18. HRMS (FAB): calcd. for $\text{C}_{25}\text{H}_{37}\text{N}_4$ (M+H-4HCl) $^+$: 393.3013; found: 393.3018

N-(4-Amino-butyl)-N-{3-[(anthracen-9-ylmethyl)-amino]-propyl}-butane-1,4-diamine Tetrahydrochloride (13c) Bright yellow solid; yield 95%; ^1H NMR : δ 8.80 (s, 1H), 8.40 (d, 2H), 8.19 (d, 2H), 7.66 (t, 2H), 7.60 (t, 2H), 5.21 (s, 2H), 3.40 (t, 2H), 3.02(t, 2H), 2.92(m, 6H), 2.82(t, 2H), 2.11 (m, 2H), 1.62(m, 8H). ^{13}C NMR: δ 130.85, 130.62, 130.29, 129.57, 127.86, 125.64, 122.61, 120.38, 47.26, 47.18, 47.10, 44.86, 44.72, 43.25, 39.06, 24.23, 23.07(2C), 22.99. Anal. Calcd. for $\text{C}_{26}\text{H}_{42}\text{Cl}_4\text{N}_4 \cdot 1.2\text{H}_2\text{O}$: C, 54.40; H, 7.80; N, 9.76. found: C, 54.43; H, 7.67; N, 9.79. HRMS (FAB): calcd. for $\text{C}_{26}\text{H}_{39}\text{N}_4$ (M+H-4HCl) $^+$: 407.3175; found: 407.3165

N-(4-Amino-butyl)-N-{3-[(anthracen-9-ylmethyl)-amino]-propyl}-pentane-1,5-diamine Tetrahydrochloride (13d) Bright yellow solid; yield 88%; ^1H NMR : δ 8.70 (s, 1H), 8.28 (d, 2H), 8.15 (d, 2H), 7.63 (t, 2H), 7.58 (t, 2H), 5.20 (s, 2H), 3.38 (t, 2H), 3.01(t, 2H), 2.90(m, 8H), 2.11 (m, 2H), 1.62(br s, 8H), 1.28(m, 2H). ^{13}C NMR: δ 130.71, 130.52, 130.14, 129.53, 127.80, 125.57, 122.55, 120.16, 47.70, 47.54, 47.12, 44.84, 44.65, 43.13, 39.10, 25.39(2C), 24.26, 23.16, 23.08, 22.97. Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{Cl}_4\text{N}_4 \cdot 1.0\text{H}_2\text{O}$: C, 55.48; H, 7.93; N, 9.59. found: C, 55.52; H, 7.97; N, 9.56. HRMS (FAB): calcd. for $\text{C}_{27}\text{H}_{41}\text{N}_4$ (M+H-4HCl) $^+$: 421.3326; found: 421.3327

N-[4-(3-Amino-propylamino)-butyl]-N-anthracen-9-ylmethyl-butane-1,4-diamine Tetrahydrochloride (13e) Bright yellow solid; yield 94%; ^1H NMR : δ 8.79 (s, 1H),

8.40 (d, 2H), 8.19 (d, 2H), 7.68 (t, 2H), 7.60 (t, 2H), 5.21 (s, 2H), 3.28 (t, 2H), 2.92(m, 10H), 1.92 (m, 2H), 1.80(m, 2H), 1.70(br s, 6H). ^{13}C NMR: δ 130.69, 130.41, 130.09, 129.49, 127.73, 125.53, 122.52, 120.37, 47.28, 47.20, 47.12, 47.06, 44.81, 42.82, 36.82, 24.05, 23.15, 23.08(2C), 23.01. Anal. Calcd. for $\text{C}_{26}\text{H}_{42}\text{Cl}_4\text{N}_4 \cdot 0.5\text{H}_2\text{O}$: C, 55.62; H, 7.72; N, 9.98. found: C, 55.62; H, 7.55; N, 9.85. HRMS (FAB): calcd. for $\text{C}_{26}\text{H}_{39}\text{N}_4$ (M+H-4HCl) $^+$: 407.3169; found: 407.3166

N-[4-(4-Amino-butylamino)-butyl]-N-anthracen-9-ylmethyl-butane-1,4-diamine

Tetrahydrochloride (13f) Bright yellow solid; yield 86%; ^1H NMR : δ 8.79 (s, 1H), 8.40 (d, 2H), 8.19 (d, 2H), 7.68 (t, 2H), 7.60 (t, 2H), 5.21 (s, 2H), 3.24 (br s, 2H), 2.92(br s, 8H), 2.84(br s, 2H), 1.80 (br s, 2H), 1.64(br s, 10H). ^{13}C NMR: δ 130.75, 130.46, 130.14, 129.53, 127.77, 125.57, 122.57, 120.45, 47.24, 47.20, 47.14(2C), 47.08, 42.88, 39.09, 24.26, 23.18, 23.12(2C), 23.10, 23.06. Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{Cl}_4\text{N}_4 \cdot 0.2\text{H}_2\text{O}$: C, 56.89; H, 7.85; N, 9.83. found: C, 56.82; H, 7.87; N, 9.76. HRMS (FAB): calcd. for $\text{C}_{27}\text{H}_{41}\text{N}_4$ (M+H-4HCl) $^+$: 421.3326; found: 421.3310

N-[4-(3-Amino-propylamino)-butyl]-N-anthracen-9-ylmethyl-pentane-1,5-diamine

Tetrahydrochloride (13g) Bright yellow solid; yield 88%; ^1H NMR : δ 8.79 (s, 1H), 8.40 (d, 2H), 8.19 (d, 2H), 7.66 (t, 2H), 7.60 (t, 2H), 5.20 (s, 2H), 3.22 (t, 2H), 2.90(m, 10H), 1.92 (m, 2H), 1.66(m, 8H), 1.40(m, 2H). ^{13}C NMR: δ 130.70, 130.36, 130.07, 129.49, 127.72, 125.54, 122.53, 120.50, 47.64, 47.53, 47.30, 47.05, 44.82, 42.69, 36.84, 25.36, 25.28, 24.07, 23.27, 23.11, 23.09. Anal. Calcd. for $\text{C}_{27}\text{H}_{44}\text{Cl}_4\text{N}_4 \cdot 0.5\text{H}_2\text{O}$: C, 56.35; H, 7.88; N, 9.74. found: C, 56.36; H, 7.76; N, 9.77. HRMS (FAB): calcd. for $\text{C}_{27}\text{H}_{41}\text{N}_4$ (M+H-4HCl) $^+$: 421.3326; found: 421.3319

N-[4-(4-Amino-butylamino)-butyl]-N-anthracen-9-ylmethyl-pentane-1,5-diamine

Tetrahydrochloride (13h) Bright yellow solid; yield 92%; ^1H NMR : δ 8.78 (s, 1H), 8.39 (d, 2H), 8.18 (d, 2H), 7.66 (t, 2H), 7.60 (t, 2H), 5.20 (s, 2H), 3.22 (t, 2H), 2.88(br s, 8H), 2.80(m, 2H), 1.74 (m, 2H), 1.62(br s, 10H), 1.40(m, 2H). ^{13}C NMR: δ 130.70, 130.36, 130.07, 129.49, 127.72, 125.54, 122.53, 120.50, 47.64, 47.53, 47.20, 47.14, 47.06, 42.70, 39.08, 25.36, 25.28, 24.24, 23.27, 23.12(2C), 23.08. Anal. Calcd. for $\text{C}_{28}\text{H}_{46}\text{Cl}_4\text{N}_4 \cdot 0.6\text{H}_2\text{O}$: C, 56.88; H, 8.05; N, 9.48. found: C, 56.89; H, 7.92; N, 9.40. HRMS(FAB): calcd. for $\text{C}_{28}\text{H}_{43}\text{N}_4 (\text{M}+\text{H}-4\text{HCl})^+$: 435.3482; found: 435.3477.

N-[5-(4-Amino-butylamino)-pentyl]-N-anthracen-9-ylmethyl-pentane-1,5-diamine

Tetrahydrochloride (13i) Bright yellow solid; yield 99%; ^1H NMR : δ 8.70 (s, 1H), 8.28 (d, 2H), 8.13 (d, 2H), 7.66 (t, 2H), 7.56 (t, 2H), 5.24 (s, 2H), 3.16 (t, 2H), 2.88(m, 10H), 1.70 (m, 2H), 1.58(m, 10H), 1.32(m, 4H). ^{13}C NMR: δ 130.69, 130.35, 130.06, 129.49, 127.70, 125.52, 122.52, 120.50, 47.62, 47.56, 47.48, 47.44, 47.11, 42.67, 39.08, 25.42(2C), 25.34, 25.27, 24.25, 23.27, 23.21, 23.07. Anal. Calcd. for $\text{C}_{29}\text{H}_{48}\text{Cl}_4\text{N}_4 \cdot 0.7\text{H}_2\text{O}$: C, 57.37; H, 8.20; N, 9.23. found: C, 57.27; H, 8.11; N, 9.14. HRMS (FAB): calcd. for $\text{C}_{29}\text{H}_{45}\text{N}_4 (\text{M}+\text{H}-4\text{HCl})^+$: 449.3644; found: 449.3650.

Synthesis of 23. A mixture of doxorubicin **21** (1 equiv.) and {4-[tert-butoxycarbonyl-(4-oxo-butyl)-amino]-butyl}-carbamic acid tert-butyl ester **20** (2 equiv.) in 25% methanol-dichloromethane (5 mL) were stirred at 0°C for several minutes, then added 1M NaBH₃CN in THF (0.67 equiv.) (Figure 5). After checking the disappearance of doxorubicin by TLC and a new less polar spot formed, the solution was concentrated and the residue was purified by preparative TLC. R_f =0.38 (10% methanol-chloroform) to give **23**. Compound **23**: ^1H NMR (CDCl_3) δ 8.05 (d, 1H, H1), 7.80 (t, 1H, H2), 7.41 (d, 1H,

H3), 5.52 (m, 1H, H1'), 5.33 (m, 1H, H7), 4.77 (m, 2H, H14), 4.58 (m, 1H, NHCO), 4.10 (s, 3H, OCH₃), 3.98 (q, 1H, H5'), 3.61 (m, 1H, H4'), 3.29 (d, 1H, H10), 3.22-3.02 (m, 7H, H10, 3xCH₂), 2.79 (m, 1H, H3'), 2.59 (m, 2H, NCH₂), 2.40 (d, 1H, H8), 2.16 (dd, 1H, H8), 1.75 (m, 1H, H2'), 1.70-1.35 (m, 30H, H2', 8xCH₂, 6xCH₃).

5 **4-(N-Benzylamino)-butan-1-ol (24a)**. Pale yellow liquid; yield 89%; $R_f=0.34$, methanol/chloroform, 1:4); ¹H NMR (300 MHz, CDCl₃) δ 7.29 (brs, 5H), 3.77 (s, 2H), 3.59 (t, 2H), 2.68 (t, 2H), 1.65 (brs, 4H); ¹³C NMR: δ 138.8, 128.4 (2C), 128.1 (2C), 127.1, 62.5, 53.7, 49.1, 32.4, 28.5; HRMS (FAB) m/z calcd. for C₁₁H₁₈NO(M+H)⁺: 180.1388; found: 180.1389.

10 **N-(Naphthalen-1-ylmethyl)-4-amino-butan-1-ol (24b)**. Dark yellow liquid; yield 93.6%; $R_f=0.54$, methanol/chloroform, 1:4); ¹H NMR (300 MHz, CDCl₃) δ 8.02 (d, 1H), 7.85 (d, 1H), 7.76 (d, 1H), 7.47 (m, 4H), 4.23 (s, 2H), 3.58 (t, 2H), 2.79 (t, 2H), 2.51 (brs, 2H), 1.68 (brs, 4H); ¹³C NMR: δ 134.5, 133.6, 131.3, 128.6, 127.8, 126.2, 126.1, 125.5, 125.2, 122.9, 62.4, 51.0, 49.6, 32.1, 28.3; HRMS m/z calcd. for C₁₅H₁₉NO(M⁺): 229.1467; found: 229.1477.

4-[(Anthracen-9-ylmethyl)-amino]-butan-1-ol (24c). See 4c above.

4-[(Pyren-1-ylmethyl)-amino]-butan-1-ol (24d). White solid; yield 94%. ¹H NMR (300 MHz, CDCl₃): δ 8.24 (d, 2H), 8.13 (m, 4H), 7.96 (m, 4H), 4.42 (s, 2H), 3.56 (t, 2H), 2.82 (t, 2H), 1.64 (brs, 4H). ¹³C NMR (CDCl₃): 132.4, 131.0, 130.6, 130.5, 128.7, 127.7, 127.2, 127.0 (2C), 125.7, 125.0, 124.9, 124.8, 124.6 (2C), 122.4, 62.6, 51.3, 49.6, 32.3, 28.6. HRMS (FAB): calcd. for C₂₁H₂₂NO(M+H)⁺: 304.1701; found: 304.1701.

N-(4-Amino-butyl)-N'-benzene-1-ylmethyl-butane-1,4-diamine Trihydrochloride (27a).

White solid; yield 73%. ¹H NMR (300 MHz, DMSO-D₂O): δ 7.47 (br s, 2H), 7.41 (br s,

3H), 4.09 (s, 2H), 2.88 (br s, 6H), 2.78 (br s, 2H), 1.63 (br s, 8H). ^{13}C NMR (D_2O): 130.7, 129.9 (2C), 129.8, 129.4 (2C), 51.3, 47.13, 47.07, 46.5, 39.0, 24.2, 23.1, 23.0 (2C); HRMS (FAB): calcd. for $\text{C}_{15}\text{H}_{28}\text{N}_3(\text{M}+\text{H}-3\text{HCl})^+$: 250.2283; found: 250.2268.

N-(4-Amino-butyl)-N'-naphthalen-1-ylmethyl-butane-1,4-diamine Trihydrochloride

5 **(27b)**. White solid; yield 80%. ^1H NMR (300 MHz, D_2O): δ 8.03 (br s, 1H), 7.96 (br s, 2H), 7.59 (br s, 2H), 7.53 (br s, 1H), 7.51 (br s, 1H), 4.68 (s, 2H), 3.14 (t, 2H), 2.99 (m, 6H), 1.67 (br s, 8). ^{13}C NMR (D_2O): 132.9, 130.2, 129.9, 128.8, 128.5, 126.8, 126.0, 125.8, 125.0, 121.9, 47.4, 46.5, 46.4, 46.3, 38.4, 23.5, 22.5, 22.4 (2C); HRMS(FAB): calcd. for $\text{C}_{19}\text{H}_{30}\text{N}_3(\text{M}+\text{H}-3\text{HCl})^+$: 300.2440; found: 300.2431.

10 **N-(4-Amino-butyl)-N'-pyren-1-ylmethyl-butane-1,4-diamine Trihydrochloride**

(27d). White solid; yield 94%. ^1H NMR (300 MHz, D_2O): δ 8.1~7.6 (m, 9H), 4.46 (s, 2H), 3.04 (m, 2H), 2.92 (m, 6H), 1.63 (br s, 8H). ^{13}C NMR (D_2O): δ 131.3, 130.4, 129.7, 128.4, 128.2, 127.9, 126.9, 126.3, 125.7, 125.6, 124.53, 124.51, 123.1, 122.9, 122.5, 120.9, 47.9, 47.0, 46.9, 46.6, 39.0, 24.1, 23.0, 22.9 (2C). HRMS (FAB): calcd. for

15 $\text{C}_{25}\text{H}_{31}\text{N}_3(\text{M}+\text{H}-3\text{HCl})^+$: 374.2596; found: 374.2594.

1-Amino-3-{4-[(anthracen-9-ylmethyl)-amino]-butylamino}-propan-2-ol

Trihydrochloride (30) Bright yellow solid, yield 95%. ^1H NMR ($\text{DMSO}-d_6 + \text{D}_2\text{O}$): δ 8.80 (s, 1H), 8.42 (d, 2H), 8.20 (d, 2H), 7.68 (t, 2H), 7.60 (t, 2H), 5.22 (s, 2H), 4.20(m, 1H), 3.28 (t, 2H), 3.1~2.80 (m, 6H), 1.78 (brs, 4H). ^{13}C NMR (D_2O): δ 130.6, 130.4, 130.0, 129.5, 127.7, 125.5, 122.5, 120.3, 63.8, 50.1, 47.3, 47.2, 42.8, 42.4, 23.0, 22.9. HRMS (FAB): calcd. for $\text{C}_{22}\text{H}_{30}\text{N}_3\text{O}(\text{M}+\text{H}-3\text{HCl})^+$: 352.2389; found: 352.2381.

N-{4-[(Anthracen-9-ylmethyl)-amino]-butyl}-cyclohexane-1,4-diamine

Trihydrochloride (31) . Bright yellow solid, yield 95%. ^1H NMR (CD_3OD): δ 8.68 (s,

1H), 8.41 (d, 2H), 8.17 (d, 2H), 7.68 (t, 2H), 7.57 (t, 2H), 5.34 (s, 2H), 3.40 (m, 2H), 3.16 (m, 4H), 2.26(m, 2H), 2.20(m, 2H), 1.90(m, 4H), 1.60(m, 4H); ¹³C NMR(D₂O): δ 130.7, 130.4, 130.1, 129.5, 127.7, 125.5, 122.5, 120.3, 55.4, 48.7, 47.1, 44.3, 42.8, 28.2, 26.8, 23.3, 23.0. HRMS (FAB): calcd. for C₂₅H₃₄N₃ (M+H-3HCl)⁺: 376.2753; found:
5 376.2747.

N¹-Anthracen-9-ylmethyl-octane-1,8-diamine Dihydrochloride (32). yellow solid; yield 89%; ¹H NMR (300 MHz, CD₃OD) δ 8.69 (s, 1H), 8.40 (d, 2H), 8.15 (d, 2H), 7.70 (t, 2H), 7.59 (t, 2H), 5.32 (s, 2H), 3.30 (br s, 2H), 2.92 (t 2H), 1.82 (m, 2H), 1.63 (m, 2H), 1.42 (br s, 8H); ¹³C NMR (D₂O): δ 130.6, 130.3, 130.0, 129.4, 127.6, 125.5, 122.4, 120.5,
10 47.8, 42.3, 39.7, 28.2, 28.1, 26.9, 25.9, 25.7, 25.4; HRMS (FAB) m/z calcd. for C₂₃H₃₁N₂ (M+H-2HCl)⁺: 335.2487; found: 335.2489.

2-(2-{2-[(Anthracen-9-ylmethyl)-amino]-ethoxy}-ethoxy)-ethylamine Dihydrochloride (33). Bright yellow solid; yield 81%; ¹H NMR (300 MHz, DMSO-d₆ +D₂O) δ 8.80 (s, 1H), 8.40 (d, 2H), 8.20 (d, 2H), 7.68 (t, 2H), 7.60 (t, 2H), 5.24 (s, 2H),
15 3.82 (t, 2H), 3.66 (m, 4H), 3.60 (t, 2H), 3.40 (t, 2H), 2.96 (t, 2H); ¹³C NMR(D₂O): δ 130.8, 130.5, 130.1, 129.5, 127.7, 125.6, 122.5, 120.5, 69.8 (2C), 66.6, 65.1, 47.0, 42.5, 39.2. HRMS (FAB) m/z calcd. for: C₂₁H₂₇N₂O₂ (M+H-2HCl)⁺: 339.2073; found: 339.2074.

N¹-Anthracen-9-ylmethyl-butane-1,4-diamine Dihydrochloride (34). Yellow solid; yield 21%; R_f=0.11, methanol/chloroform, 1:20 + 3 drops of NH₄OH); ¹H NMR (300 MHz, D₂O): δ 8.55 (br s, 1H), 8.15 (d, 2H), 8.08 (d, 2H), 7.66 (m, 4H), 5.08 (br s, 2H),
20 3.25 (t, 2H), 3.0 (t, 2H), 1.76 (m, 4H); ¹³C NMR (D₂O): δ 130.6, 130.3, 129.9, 129.4,

127.6, 125.4, 122.4, 120.2, 47.2, 42.7, 38.6, 24.2, 22.9; ESI-MS m/z calcd. for $C_{19}H_{23}N_2$ (M+H): 279.2; found: 279.2.

N¹-Anthracen-9-ylmethyl-butylamine Monohydrochloride (35) Compound **35** was synthesized in 58% yield by reductive amination of anthraldehyde and butylamine followed by treatment with 4N aq. HCl. **35**: Yellow solid; yield 58%; R_f = 0.5, methanol/chloroform, 1:20 + 1 drop of NH_4OH ; 1H NMR (300 MHz, DMSO- d_6): δ 9.1 (br s, 2H, NH_2 salt), 8.78 (s, 1H), 8.51 (d, 2H), 8.18 (d, 2H), 7.64 (m, 4H), 5.2 (br s, 2H), 3.2 (s, 2H), 1.73 (t, 2H), 1.36 (q, 2H), 0.92 (q, 3H); ^{13}C NMR ($CDCl_3$): δ 131.6, 131.26, 130.55, 129.46, 128.05, 125.72, 123.89, 120.86, 46.05, 41.92, 28.48, 20.32, 13.79. Anal. Calcd for $C_{15}H_{22}NCl \cdot 0.2H_2O$: C, 75.21; H, 7.44; N, 4.62; found: C, 75.31; H, 7.44; N, 4.56.

As is apparent from the above Tables 1-3, a limited class of *N*-alkylaryl polyamine compounds (i.e. *N*-naphthylalkyl, *N*-anthracenylalkyl and *N*-pyrenylalkyl) have unique properties of surprising cytotoxicity, unexpected selectivity in killing cancer cells (especially cells with high polyamine transport activity), and/or facilitate the delivery of known toxic agents into cancer cells. As shown in Table 2, the *N*-(3-Amino-propyl)-*N*-anthracen-9-ylmethyl-butane-1,4-diamine, trihydrochloride (**8d**), *N*-(4-Amino-butyl)-*N*-anthracen-9-ylmethyl-butane-1,4-diamine, trihydrochloride (**8e**), *N*-(4-Amino-butyl)-*N*-anthracen-9-ylmethyl-pentane-1,5-diamine, trihydrochloride (**8g**), *N*-(4-Amino-butyl)-*N*-naphthalen-1-ylmethyl-butane-1,4-diamine trihydrochloride (**27b**), *N*-(4-Amino-butyl)-*N*-pyren-1-ylmethyl-butane-1,4-diamine trihydrochloride (**27d**), and *N*-{4-[(Anthracen-9-ylmethyl)-amino]-butyl}-cyclohexane-1,4-diamine trihydrochloride (**31**) have outstanding selectivity in targeting and killing cells with active polyamine transporters.

N-Alkylpolyamines can be broken down into their de-alkylated components by cellular metabolic pathways. For example, a *N*-alkyl-4,4-triamine ($\text{RNH}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_4\text{NH}_2$, where R is alkyl) can be converted into homospermidine, $\text{H}_2\text{N}(\text{CH}_2)_4\text{NH}(\text{CH}_2)_4\text{NH}_2$. It has been found that the *N*-alkylaryl group has an influence
5 on this breakdown pathway. Surprisingly, cells treated with compound **8e** did not form homospermidine, which indicated stability towards this form of degradation.

While the invention has been described, disclosed, illustrated and shown in various terms of certain embodiments or modifications which it has presumed in practice, the scope of the invention is not intended to be, nor should it be deemed to be, limited
10 thereby and such other modifications or embodiments as may be suggested by the teachings herein are particularly reserved especially as they fall within the breadth and scope of the claims here appended.